Quantibody® Mouse Cytokine Antibody Array 4000

--Quantitative measurement of 200 mouse cytokines

Patent Pending Technology

User Manual (Version Dec 2013)

Quantibody® Mouse Cytokine Antibody Array 4000

(Combination of Quantibody® Mouse Cytokine Array 4, Mouse Cytokine Array 5, Mouse Cytokine Array 6, Mouse Cytokine Array 7 and Mouse Cytokine Array 8 to quantitatively measure the concentration of 200 mouse cytokines)

Cat # QAM-CAA-4000

Quantibody® Mouse Cytokine Array 4 (Cat# QAM-CYT-4)
Quantibody® Mouse Cytokine Array 5 (Cat# QAM-CYT-5)
Quantibody® Mouse Cytokine Array 6 (Cat# QAM-CYT-6)
Quantibody® Mouse Cytokine Array 7 (Cat# QAM-CYT-7)
Quantibody® Mouse Cytokine Array 8 (Cat# QAM-CYT-8)

RayBiotech, Inc.

We Provide You With Excellent Protein Array Systems and Service

Tel:(Toll Free) 1-888-494-8555 or 770-729-2992; Fax: 1-888-547-0580;
Website:www.raybiotech.com Email: info@raybiotech.com

Cytokine Detected	200
Quantibody® Mouse Cytokine Array 4 (40)	Axl, CD27L, CD30T, CD40, CXCL16, EGF, E-selectin, Fractalkine, GITR, HGF, IGFBP-2, IGFBP-3, IGFBP-5, IGFBP-6, IGF-I, IL-12p70, IL-17E, IL-17F, IL-1ra, IL-2 Rα, IL-20, IL-23, IL-28, I-TAC, MDC, MIP-2, MIP-3α, OPN, OPG, Prolactin, Pro-MMP-9, P-selectin, Resistin, SCF, SDF-1α, TPO, VCAM-1, VEGF, VEGF-D
Quantibody® Mouse Cytokine Array 5 (40)	bFGF, BLC, CD30L, Eotaxin, Eotaxin-2, Fas L, G-CSF, GM-CSF, ICAM-1, IFNγ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p40, IL-13, IL-15, IL-17, IL-21, KC, Leptin, LIX, MCP-1, MCP-5, M-CSF, MIG, MIP-1α, MIP-1γ, PF-4, RANTES, TARC, TCA-3, TNFα, TNFRI, TNFRII
Quantibody® Mouse Cytokine Array 6 (40)	4-1BB, ACE, ALK-1, CT-1, CD27, CD40L, CTLA-4, Decorin, Dkk-1, Dtk, Endoglin, Fcγ RIIB, Flt-3 L, Galectin-1, Galectin-3, Gas 1, Gas 6, GITR L, HAI-1, HGF R, IL-1 R4, IL-3 Rβ, IL-9, JAM-A, Leptin R, L-Selectin, Lymphotactin, MadCAM-1, MFG-E8, MIP-3β, Neprilysin, Pentraxin 3, RAGE, TACI, TREM-1, TROY, TSLP, TWEAK R, VEGF R1, VEGF R3
Quantibody® Mouse Cytokine Array 7 (40)	B7-1, BAFF R, BTC, C5a, CCL6, CD48, CD6, Chemerin, Clusterin, CXCL15, Cystatin C, DAN, DLL4, EDAR, Endocan, Fetuin A, H60, IL-33, IL-7 Ralpha, Kremen-1, Limitin, Lipocalin-2, LOX-1, Marapsin, MBL-2, Meteorin, Nope, NOV, Osteoactivin, OX40, Ligand, P-Cadherin, Periostin, PlGF-2, Progranulin, Prostasin, Renin 1, Testican 3, TIM-1, TRAIL, Tryptase epsilon
Quantibody® Mouse Cytokine Array 8 (40)	6Ckine, Activin A, ADAMTS1, Adiponectin, ANG-3, ANGPTL3, Artemin, CCL28, CD36, Chordin, CRP, E-Cadherin, Epigen, Epiregulin, Fas, Galectin-7, gp130, Granzyme B, Gremlin, IFN-γ R1, IL-17B, IL-17B R, IL-22, MIP-1β, MMP-2, MMP-3, MMP-10, PDGF-AA, Persephin, sFRP-3, Shh-N, SLAM, TCK-1, TECK, TGFβ1, TRANCE, TremL1, TWEAK, VEGF-B, VEGF-R2
Format	One standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody is arrayed in quadruplicate.
Detection Method	Fluorescence with laser scanner: Cy3 equivalent dye
Sample Volume	50 – 100 µl per array
Reproducibility	CV <20%
Assay duration	6 hrs

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I. Introduction

Cytokines play an important role in innate immunity, apoptosis, angiogenesis, cell growth and differentiation. They are involved in interactions between different cell types, cellular responses to environmental conditions, and maintenance of homeostasis. In addition, cytokines are also involved in most disease processes, including cancer and cardiac diseases.

The traditional method for cytokine detection and quantification is through the use of an enzyme-linked immunosorbent array (ELISA). In this method, a target protein is first immobilized to a solid support. The immobilized protein is then complexed with an antibody that is linked to an enzyme. Detection of the enzyme-complex can then be visualized through the use of a substrate that produces a detectable signal. While this traditional method works well for a single protein, the overall procedure is time consuming and requires a relatively high volume of sample. Thus, conservation of precious small sample quantities becomes a risky task. To solve this problem take advantage of the innovations in microarray technology over the last decade. A long-standing leader in the field, Raybiotech, has pioneered the development of cytokine antibody arrays, which have now been widely applied in the research community with hundreds of peer reviewed publications including top tier journals, such as in Cell and Nature.

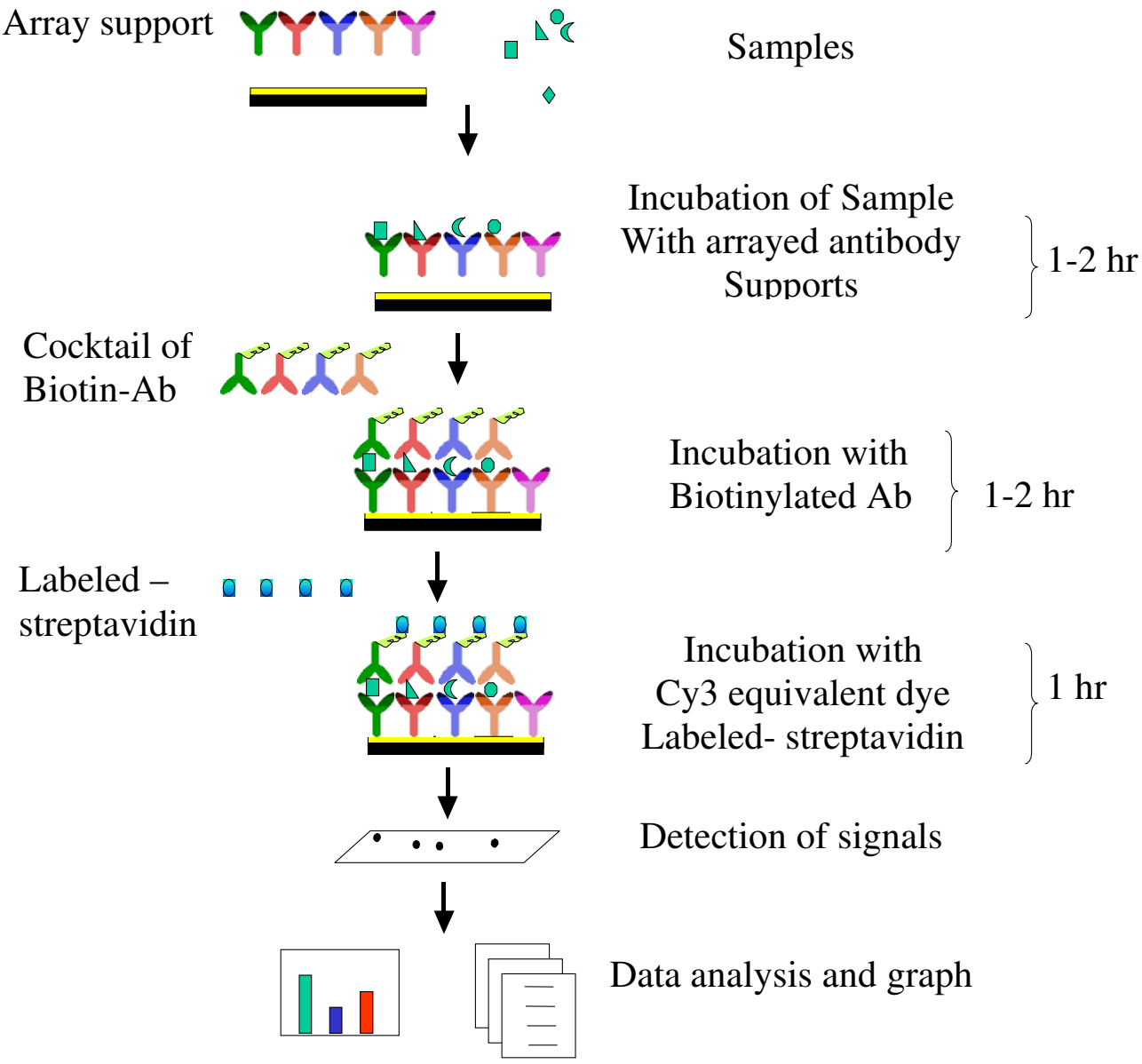
The Quantibody® array, our quantitative array platform, uses the multiplexed sandwich ELISA-based technology and enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity & specificity of ELISA and the high throughput of arrays. Like a traditional sandwich-based ELISA, it uses a pair of cytokine specific antibodies for detection. A capture antibody is first bound to the glass surface. After incubation with the sample, the target cytokine is trapped on the solid surface. A second biotin-labeled detection antibody is then added, which can recognize a different isotope of the target cytokine. The cytokine-antibody-biotin complex can then be visualized through the addition of the streptavidin-labeled Cy3 equivalent dye using a laser scanner. Unlike the traditional ELISA, Quantibody products use array format. By arraying multiple cytokine specific capture antibodies

onto a glass support, quantitative, multiplex detection of cytokines in one experiment is made possible.

In detail, one standard glass slide is divided into 16 wells of identical cytokine antibody arrays. Each antibody, together with the positive controls is arrayed in quadruplicate. The slide comes with a 16-well removable gasket which allows for the process of 16 samples on one slide. Four slides can be nested into a tray, which matches a standard microplate footprint and allows for automated robotic high throughput process of 64 arrays simultaneously. For cytokine quantification, the array specific cytokine standards, whose concentration has been predetermined, are provided to generate a standard curve for each cytokine. In a real experiment, standard cytokines and samples will be assayed in each array simultaneously through a sandwich ELISA procedure. By comparing signals from unknown samples to the standard curve, the cytokine concentration in the samples will be determined.

Quantibody® array kits have been confirmed to have similar detection sensitivity as traditional ELISA. Our current high density Quantibody kits allow scientists to quantitatively determine the concentration of 400 human 200 mouse, or 100 rat cytokines in a single experiment. This is not only one of the most efficient products on the market for cytokine quantification, but makes it more affordable for quantification of large number of proteins. Simultaneous detection of multiple cytokines undoubtedly provides a powerful tool for drug and biomarker discovery.

How It Works


Array support
Samples
Incubation of Sample
With arrayed antibody
Supports
1-2 hr
Cocktail of
Biotin-Ab
Incubation with
Biotinylated Ab
1-2 hr
Labeled –
streptavidin
Incubation with
Cy3 equivalent dye
Labeled- streptavidin
1 hr
Detection of signals
Data analysis and graph

II. Materials Provided

Upon receipt, all components of the Quantibody® Array kit should be stored at -20^{0}C. At -20^{0}C the kit will retain complete activity for up to 6 months. Once thawed, the glass slide, cytokine standard mix, detection antibody cocktail and Cy3 equivalent dye-conjugated Streptavidin should be kept at –20^{0}C and all other components may be stored at 4^{0}C. The entire kit should be used within 6 months of purchase.

Components

Item	Description	QAM-CCA-4000-1	QAM-CCA-4000-2
1	Quantibody® Array Glass Slide	5	10
2	Sample Diluent	5	5
3	20X Wash Buffer I	10	15
4	20X Wash Buffer II	5	5
5	Lyophilized cytokine standard mix	5	10
6	Detection antibody cocktail	5	10
7	Cy3 equivalent dye-conjugated Streptavidin	5	10
8	Slide Washer/Dryer	5	5
9	Adhesive device sealer	25	50
10	Manual	5	5

* The independent sets of reagents for Quantibody® Mouse Cytokine Array 4, 5, 6, 7, and 8 were shipped in three different boxes. Among all the reagents, the glass slide, lyophilized cytokine standard mix, and detection antibody cocktail are array specific, while all other reagents are interchangeable between the arrays.

Note: See Section VI for detailed cytokine concentrations after reconstitution.

Additional Materials Required

- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- ddH_2O
- 1.5ml Polypropylene microcentrifuge tubes

III. General Considerations

A. Preparation of Samples

- Use serum-free conditioned media if possible.
- If serum-containing conditioned media is required, it is highly recommended that complete medium be used as a control since many types of sera contains cytokines.
- We recommend the following parameters for your samples:
  50 to 100 µl of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates.

  If you experience high background or the readings exceed the detection range, further dilution of your sample is recommended.

B. Handling glass slides

- Do not touch the surface of the slides, as the microarray slides are very sensitive. Hold the slides by the edges only.
- Handle all buffers and slides with latex free gloves.
- Handle glass slide in clean environment.
- Because there is no barcode on the slide, transcribe the slide serial number from the slide bag to the back of the slide with a permanent marker before discarding the slide bag. Once the slide is disassembled, you might not have enough info to distinguish one slide from the other.

C. Incubation

- Completely cover array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or <70 µl of sample or reagent is used.
- Several incubation steps such as step 7 (blocking and incubation), step 10 (detection antibody incubation), or step 13 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at 4^0C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

IV. Protocol

READ ENTIRE PROTOCOL BEFORE STARTING

Note: There are nine sets of reagents for the nine ***different arrays.*** *Be careful to use the correct glass slide, lyophilized cytokine standard, and the detection antibody cocktail for the corresponding array. The following is the procedure for processing any one of the arrays in the kit.*

A. Completely air dry the glass slide

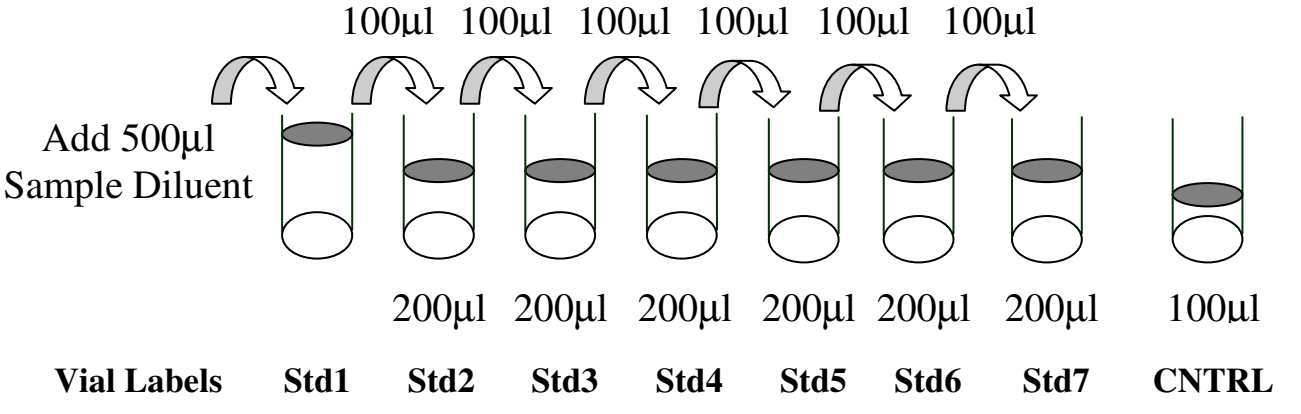
1. Take out the glass slide from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

Note: Incomplete drying of slides before use may cause the formation of streaks or "comet tails" on a slide.

B. Prepare Cytokine Standard Dilutions

Note: There is only one vial of standard provided in the two-slide kit, this is enough for making two standard curves. Reconstitute the lyophilized standard within one hour of usage. If you must use the standard for two different days, store only the Std1 dilution at -80 ^{0}C for future use.

Prepare serial dilution of cytokine standards


100µl 100µl 100µl 100µl 100µl 100µl
Add 500µl
Sample Diluent
200µl 200µl 200µl 200µl 200µl 200µl 100µl
Vial Labels Std1 Std2 Std3 Std4 Std5 Std6 Std7 CNTRL

2. Reconstitute the Cytokine Standard Mix (lyophilized) by adding 500µl Sample Diluent to the tube. For best recovery, always quick-spin vial prior to opening. Dissolve the powder thoroughly by a gentle mix. Labeled the tube as Std1.

3. Label 6 clean microcentrifuge tubes as Std2 to Std7. Add 200µl Sample Diluent to each of the tubes.

4. Pipette 100µl Std1 into tube Std2 and mix gently. Perform 5 more serial dilutions by adding 100ul Std2 to tube Std3 and so on.

5. Add 100µl Sample Diluent to another tube labeled as CNTRL. Do not add standard cytokines or samples to the CNTRL tube, which will be used as negative control. For best results, include a set of standards in each slide.

Note: Since the starting concentration of each cytokine is different, the serial concentrations from Std1 to Std7 for each cytokine are varied which can be found in section VI.

C. Blocking and Incubation

6. Add 100µl Sample Diluent into each well and incubate at room temperature for 30 min to block slides.

7. Decant buffer from each well. Add 100µl standard cytokines or samples to each well. Incubate arrays at room temperature for 1-2 hour. *(Be careful to use the corresponding cytokine standard for the matching glass slide.)*

   Note: We recommend using 50 to 100 µl of original or diluted serum, plasma, conditioned media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation if less than 70 ul of sample or reagent is used.

Note: This step may be done overnight at 4^0C for best results. Longer incubation time is preferable for higher signal.

8. Wash:
   - Calculate the volumes of Wash Buffers required based on the number of samples being processed and the entire remaining protocol described below.
   - Dilute 20x Wash Buffer I and 20x Wash Buffer II separately with ddH_2O to generate the required volume of 1x Wash Buffer I and 1x Wash Buffer II. For example 100 µl of 20x Wash Buffer I would be diluted to a final volume of 2,000 µl.
   - Decant the samples from each well, and wash 5 times (5 min each) with 150 µl of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer after each wash step.
   - (*Optional for Cell and Tissue Lysates*) Put the glass slide with frame into a box with 1x Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle shaking for 20 min.
   - Decant the 1x Wash Buffer I from each well, wash 2 times (5 min each) with 150 µl of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

Note: Incomplete removal of the wash buffer in each wash step may cause "dark spots". (Background signal is higher than that of the spot.)

D. Incubation with detection antibody cocktail and wash.

9. Reconstitute the detection antibody by adding 1.4 ml of Sample Diluent to the tube. Spin briefly.

10. Add 80 µl of the detection antibody cocktail to each well. Incubate at room temperature for 1-2 hour. *(Be careful to use the corresponding detection cocktail for the matching glass slide.)*

Note: incubation may be done at 4^0C for overnight.

11. Decant the samples from each well, and wash 5 times with 150 µl of 1x Wash Buffer I and then 2 times with 150 µl of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

E. Incubation with Cy3 equivalent dye -Streptavidin and wash

12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.

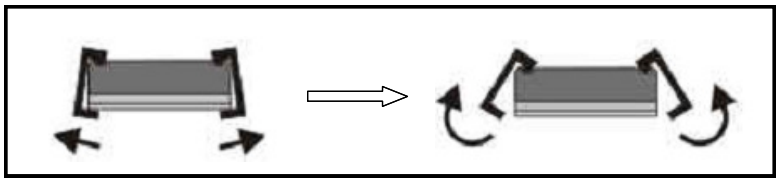
13. Add 80 µl of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the device with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at room temperature for 1 hour.

14. Decant the samples from each well, and wash 5 times with 150 µl of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

F. Fluorescence Detection

15. Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket.

(Be careful not to touch the surface of the array side)



16. Place the slide in the slide Washer/Dryer (a 4-slide holder/centrifuge tube), add enough 1x Wash Buffer I (about 30 ml) to cover the whole slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1x Wash Buffer II (about 30 ml) with gentle shaking at room temperature for 5 minutes then decant Wash Buffer II.

17. Remove water droplets completely by one of the following ways:
    - Put the glass slide into the Slide Washer/Dryer, and dry the glass slide by centrifuge at 1,000 rpm for 3 minutes without cap.
    - Or, dry the glass slide by a compressed N_2 stream.
    - Or gently apply suction with a pipette to remove water droplets. Do not touch the array, only the sides.

18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength such as Axon GenePix. Make sure that the signal from the well containing the highest standard concentration (Std1) receives the highest possible reading, yet remains unsaturated.

Note: If the signal intensity for different cytokines varies greatly in the same array, we recommend using multiple scans, with a higher PMT for low signal cytokines, and a low PMT for high signal cytokines.

G. Data Analysis

19. Data extraction can be done with most of the microarray analysis software (GenePix, ScanArray Express, ArrayVision, or MicroVigene). For quantitative data analysis, our Quantibody® Q-Analyzer software is available. It gives visual output as well as digital values. More information can be found in section VIII.

V. Cytokine Array Map

QAM-CYT-4

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	AR
b	Axl	CD27L	CD30T
c	CD40	CXCL16	EGF
d	E-selectin	Fractalkine	GITR
e	HGF	IGFBP-2	IGFBP-3
f	IGFBP-5	IGFBP-6	IGF-I
g	IL-12p70	IL-17E	IL-17F
h	IL-1ra	IL-2 Ra	IL-20
i	IL-23	IL-28	I-TAC
j	MDC	MIP-2	MIP-3a
k	OPN	OPG	Prolactin
l	Pro-MMP-9	P-selectin	Resistin
m	SCF	SDF-1a	TPO
n	VCAM-1	VEGF	VEGF-D

QAM-CYT-5

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	bFGF
b	BLC	CD30L	Eotaxin
c	Eotaxin-2	Fas L	G-CSF
d	GM-CSF	ICAM-1	IFNg
e	IL-1a	IL-1b	IL-2
f	IL-3	IL-4	IL-5
g	IL-6	IL-7	IL-10
h	IL-12p40	IL-13	IL-15
i	IL-17	IL-21	KC
j	Leptin	LIX	MCP-1
k	MCP-5	M-CSF	MIG
l	MIP-1a	MIP-1g	PF-4
m	RANTES	TARC	TCA-3
n	TNF RI	TNF RII	TNFa

QAM-CYT-6

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	4-1BB
b	ACE	ALK-1	CT-1
c	CD27	CD40L	CTLA-4
d	Decorin	Dkk-1	Dtk
e	Endoglin	Fcg RIIB	Flt-3L
f	Galectin-1	Galectin-3	Gas 1
g	Gas 6	GITR L	HAI-1
h	HGF R	IL-1 R4	IL-3 Rb
i	IL-9	JAM-A	Leptin R
j	L-Selectin	Lymphotactin	MadCAM-1
k	MFG-E8	MIP-3b	Neprilysin
l	Pentraxin 3	RAGE	TACI
m	TREM-1	TROY	TSLP
n	TWEAK R	VEGF R1	VEGF R3

QAM-CYT-7

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	B7-1
b	BAFF R	BTC	C5a
c	CCL6	CD48	CD6
d	Chemerin	Clusterin	CXCL15
e	Cystatin C	DAN	DLL4
f	EDAR	Endocan	Fetuin A
g	H60	IL-33	IL-7 Rα
h	Kremen-1	Limitin	Lipocalin-2
i	LOX-1	Marapsin	MBL-2
j	Meteorin	Nope	NOV
k	Osteoactivin	OX40 Ligand	P-Cadherin
l	Periostin	PIGF-2	Progranulin
m	Prostasin	Renin 1	Testican 3
n	TIM-1	TRAIL	Tryptase ε

QAM-CYT-8

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	6Ckine
b	Activin A	ADAMTS1	Adiponectin
c	ANG-3	ANGPTL3	Artemin
d	CCL28	CD36	Chordin
e	CRP	E-Cadherin	Epigen
f	Epiregulin	Fas	Galectin-7
g	gp130	Granzyme B	Gremlin
h	IFN-γ R1	IL-17B	IL-17B R
i	IL-22	MIP-1b	MMP-2
j	MMP-3	MMP-10	PDGF-AA
k	Persephin	sFRP-3	Shh-N
l	SLAM	TCK-1	TECK
m	TGFb1	TRANCE	TremL1
n	TWEAK	VEGF-B	VEGF-R2

VI. 8-Point Standards

After reconstitution of the lyophilized cytokine standard mix, the 8-point cytokine concentration used for generating the standard curve of a given antigen is listed below.

QAM-CYT-4 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
AR	0	3	8	25	74	222	667	2,000
Axl	0	14	41	123	370	1,111	3,333	10,000
CD27L	0	27	82	247	741	2,222	6,667	20,000
CD30T	0	14	41	123	370	1,111	3,333	10,000
CD40	0	14	41	123	370	1,111	3,333	10,000
CXCL16	0	1	4	12	37	111	333	1,000
EGF	0	3	8	25	74	222	667	2,000
E-selectin	0	5	16	49	148	444	1,333	4,000
Fractalkine	0	137	412	1,235	3,704	11,111	33,333	100,000
GITR	0	5	16	49	148	444	1,333	4,000
HGF	0	27	82	247	741	2,222	6,667	20,000
IGFBP-2	0	137	412	1,235	3,704	11,111	33,333	100,000
IGFBP-3	0	27	82	247	741	2,222	6,667	20,000
IGFBP-5	0	55	165	494	1,481	4,444	13,333	40,000
IGFBP-6	0	55	165	494	1,481	4,444	13,333	40,000
IGF-I	0	14	41	123	370	1,111	3,333	10,000
IL-12p70	0	5	16	49	148	444	1,333	4,000
IL-17E	0	55	165	494	1,481	4,444	13,333	40,000
IL-17F	0	55	165	494	1,481	4,444	13,333	40,000
IL-1ra	0	5	16	49	148	444	1,333	4,000
IL-2 Rα	0	14	41	123	370	1,111	3,333	10,000
IL-20	0	27	82	247	741	2,222	6,667	20,000
IL-23	0	55	165	494	1,481	4,444	13,333	40,000
IL-28	0	3	8	25	74	222	667	2,000
I-TAC	0	27	82	247	741	2,222	6,667	20,000
MDC	0	1	4	12	37	111	333	1,000
MIP-2	0	1	4	12	37	111	333	1,000
MIP-3α	0	1	4	12	37	111	333	1,000
OPN	0	27	82	247	741	2,222	6,667	20,000
OPG	0	27	82	247	741	2,222	6,667	20,000
Prolactin	0	14	41	123	370	1,111	3,333	10,000
Pro-MMP-9	0	137	412	1,235	3,704	11,111	33,333	100,000
P-selectin	0	5	16	49	148	444	1,333	4,000
Resistin	0	3	8	25	74	222	667	2,000
SCF	0	14	41	123	370	1,111	3,333	10,000
SDF-1α	0	137	412	1,235	3,704	11,111	33,333	100,000
TPO	0	137	412	1,235	3,704	11,111	33,333	100,000
VCAM-1	0	5	16	49	148	444	1,333	4,000
VEGF	0	5	16	49	148	444	1,333	4,000
VEGF-D	0	5	16	49	148	444	1,333	4,000

QAM-CYT-5 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
bFGF	0	7	21	62	185	556	1,667	5,000
BLC	0	14	41	123	370	1,111	3,333	10,000

CD30L	0	3	8	25	74	222	667	2,000
Eotaxin	0	1	4	12	37	111	333	1,000
Eotaxin-2	0	1	4	12	37	111	333	1,000
Fas L	0	14	41	123	370	1,111	3,333	10,000
G-CSF	0	27	82	247	741	2,222	6,667	20,000
GM-CSF	0	14	41	123	370	1,111	3,333	10,000
ICAM-1	0	14	41	123	370	1,111	3,333	10,000
IFNγ	0	5	16	49	148	444	1,333	4,000
IL-1α	0	3	8	25	74	222	667	2,000
IL-1β	0	5	16	49	148	444	1,333	4,000
IL-2	0	14	41	123	370	1,111	3,333	10,000
IL-3	0	3	8	25	74	222	667	2,000
IL-4	0	1	2	6	19	56	167	500
IL-5	0	14	41	123	370	1,111	3,333	10,000
IL-6	0	5	16	49	148	444	1,333	4,000
IL-7	0	14	41	123	370	1,111	3,333	10,000
IL-10	0	14	41	123	370	1,111	3,333	10,000
IL-12p40	0	1	4	12	37	111	333	1,000
IL-13	0	27	82	247	741	2,222	6,667	20,000
IL-15	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-17	0	5	16	49	148	444	1,333	4,000
IL-21	0	27	82	247	741	2,222	6,667	20,000
KC	0	3	8	25	74	222	667	2,000
Leptin	0	137	412	1,235	3,704	11,111	33,333	100,000
LIX	0	27	82	247	741	2,222	6,667	20,000
MCP-1	0	5	16	49	148	444	1,333	4,000
MCP-5	0	1	4	12	37	111	333	1,000
M-CSF	0	3	8	25	74	222	667	2,000
MIG	0	14	41	123	370	1,111	3,333	10,000
MIP-1α	0	14	41	123	370	1,111	3,333	10,000
MIP-1γ	0	1	4	12	37	111	333	1,000
PF-4	0	27	82	247	741	2,222	6,667	20,000
RANTES	0	5	16	49	148	444	1,333	4,000
TARC	0	5	16	49	148	444	1,333	4,000
TCA-3	0	3	8	25	74	222	667	2,000
TNF RI	0	1	2	6	19	56	167	500
TNF RII	0	3	8	25	74	222	667	2,000
TNFα	0	1	4	12	37	111	333	1,000

QAM-CYT-6 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
4-1BB	0	34	103	309	926	2,778	8,333	25,000
ACE	0	137	412	1,235	3,704	11,111	33,333	100,000
ALK-1	0	14	41	123	370	1,111	3,333	10,000
CT-1	0	55	165	494	1,481	4,444	13,333	40,000
CD27	0	34	103	309	926	2,778	8,333	25,000
CD40L	0	55	165	494	1,481	4,444	13,333	40,000
CTLA-4	0	3	10	31	93	278	833	2,500
Decorin	0	7	21	62	185	556	1,667	5,000
Dkk-1	0	55	165	494	1,481	4,444	13,333	40,000
Dtk	0	27	82	247	741	2,222	6,667	20,000
Endoglin	0	14	41	123	370	1,111	3,333	10,000
Fcγ RIIB	0	14	41	123	370	1,111	3,333	10,000
Flt-3L	0	34	103	309	926	2,778	8,333	25,000
Galectin-1	0	14	41	123	370	1,111	3,333	10,000

Galectin-3	0	3	8	25	74	222	667	2,000
Gas 1	0	3	8	25	74	222	667	2,000
Gas 6	0	3	10	31	93	278	833	2,500
GITR L	0	1	4	12	37	111	333	1,000
HAI-1	0	14	41	123	370	1,111	3,333	10,000
HGF R	0	34	103	309	926	2,778	8,333	25,000
IL-1 R4	0	55	165	494	1,481	4,444	13,333	40,000
IL-3 Rβ	0	55	165	494	1,481	4,444	13,333	40,000
IL-9	0	27	82	247	741	2,222	6,667	20,000
JAM-A	0	7	21	62	185	556	1,667	5,000
Leptin R	0	7	21	62	185	556	1,667	5,000
L-Selectin	0	14	41	123	370	1,111	3,333	10,000
Lymphotactin	0	274	823	2,469	7,407	22,222	66,667	200,000
MadCAM-1	0	14	41	123	370	1,111	3,333	10,000
MFG-E8	0	55	165	494	1,481	4,444	13,333	40,000
MIP-3β	0	1	4	12	37	111	333	1,000
Neprilysin	0	27	82	247	741	2,222	6,667	20,000
Pentraxin 3	0	14	41	123	370	1,111	3,333	10,000
RAGE	0	34	103	309	926	2,778	8,333	25,000
TACI	0	69	206	617	1,852	5,556	16,667	50,000
TREM-1	0	14	41	123	370	1,111	3,333	10,000
TROY	0	5	16	49	148	444	1,333	4,000
TSLP	0	5	16	49	148	444	1,333	4,000
TWEAK R	0	34	103	309	926	2,778	8,333	25,000
VEGF R1	0	14	41	123	370	1,111	3,333	10,000
VEGF R3	0	14	41	123	370	1,111	3,333	10,000

QAM-CYT-7 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
B7-1	0	5	16	49	148	444	1,333	4,000
BAFF R	0	1	4	12	37	111	333	1,000
BTC	0	3	8	25	74	222	667	2,000
C5a	0	1	4	12	37	111	333	1,000
CCL6	0	55	165	494	1,481	4,444	13,333	40,000
CD48	0	3	8	25	74	222	667	2,000
CD6	0	1	4	12	37	111	333	1,000
Chemerin	0	137	412	1,235	3,704	11,111	33,333	100,000
Clusterin	0	137	412	1,235	3,704	11,111	33,333	100,000
CXCL15	0	274	823	2,469	7,407	22,222	66,667	200,000
Cystatin C	0	3	8	25	74	222	667	2,000
DAN	0	137	412	1,235	3,704	11,111	33,333	100,000
DLL4	0	55	165	494	1,481	4,444	13,333	40,000
EDAR	0	27	82	247	741	2,222	6,667	20,000
Endocan	0	27	82	247	741	2,222	6,667	20,000
Fetuin A	0	137	412	1,235	3,704	11,111	33,333	100,000
H60	0	3	8	25	74	222	667	2,000
IL-33	0	5	16	49	148	444	1,333	4,000
IL-7 Rα	0	55	165	494	1,481	4,444	13,333	40,000
Kremen-1	0	5	16	49	148	444	1,333	4,000
Limitin	0	1	4	12	37	111	333	1,000
Lipocalin-2	0	137	412	1,235	3,704	11,111	33,333	100,000
LOX-1	0	5	16	49	148	444	1,333	4,000
Marapsin	0	27	82	247	741	2,222	6,667	20,000
MBL-2	0	3	8	25	74	222	667	2,000
Meteorin	0	55	165	494	1,481	4,444	13,333	40,000
Nope	0	14	41	123	370	1,111	3,333	10,000

NOV	0	55	165	494	1,481	4,444	13,333	40,000
Osteoactivin	0	14	41	123	370	1,111	3,333	10,000
OX40 Ligand	0	5	16	49	148	444	1,333	4,000
P-Cadherin	0	5	16	49	148	444	1,333	4,000
Periostin	0	5	16	49	148	444	1,333	4,000
PlGF-2	0	1	4	12	37	111	333	1,000
Progranulin	0	137	412	1,235	3,704	11,111	33,333	100,000
Prostasin	0	137	412	1,235	3,704	11,111	33,333	100,000
Renin 1	0	55	165	494	1,481	4,444	13,333	40,000
Testican 3	0	55	165	494	1,481	4,444	13,333	40,000
TIM-1	0	137	412	1,235	3,704	11,111	33,333	100,000
TRAIL	0	14	41	123	370	1,111	3,333	10,000
Tryptase ε	0	137	412	1,235	3,704	11,111	33,333	100,000

QAM-CYT-8 Serial standard concentration (pg/ml)

(pg/ml)	Control	Std7	Std6	Std5	Std4	Std3	Std2	Std1
6Ckine	0	27	82	247	741	2,222	6,667	20,000
Activin A	0	5	16	49	148	444	1,333	4,000
ADAMTS1	0	55	165	494	1,481	4,444	13,333	40,000
Adiponectin	0	14	41	123	370	1,111	3,333	10,000
ANG-3	0	55	165	494	1,481	4,444	13,333	40,000
ANGPTL3	0	137	412	1,235	3,704	11,111	33,333	100,000
Artemin	0	5	16	49	148	444	1,333	4,000
CCL28	0	137	412	1,235	3,704	11,111	33,333	100,000
CD36	0	274	823	2,469	7,407	22,222	66,667	200,000
Chordin	0	14	41	123	370	1,111	3,333	10,000
CRP	0	5	16	49	148	444	1,333	4,000
E-Cadherin	0	14	41	123	370	1,111	3,333	10,000
Epigen	0	27	82	247	741	2,222	6,667	20,000
Epiregulin	0	274	823	2,469	7,407	22,222	66,667	200,000
Fas	0	14	41	123	370	1,111	3,333	10,000
Galectin-7	0	137	412	1,235	3,704	11,111	33,333	100,000
gp130	0	14	41	123	370	1,111	3,333	10,000
Granzyme B	0	27	82	247	741	2,222	6,667	20,000
Gremlin	0	137	412	1,235	3,704	11,111	33,333	100,000
IFNγ R1	0	3	8	25	74	222	667	2,000
IL-17B	0	274	823	2,469	7,407	22,222	66,667	200,000
IL-17B R	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-22	0	55	165	494	1,481	4,444	13,333	40,000
MIP-1β	0	5	16	49	148	444	1,333	4,000
MMP-2	0	27	82	247	741	2,222	6,667	20,000
MMP-3	0	14	41	123	370	1,111	3,333	10,000
MMP-10	0	1	4	12	37	111	333	1,000
PDGF-AA	0	5	16	49	148	444	1,333	4,000
Persephin	0	5	16	49	148	444	1,333	4,000
sFRP-3	0	27	82	247	741	2,222	6,667	20,000
Shh-N	0	14	41	123	370	1,111	3,333	10,000
SLAM	0	137	412	1,235	3,704	11,111	33,333	100,000
TCK-1	0	274	823	2,469	7,407	22,222	66,667	200,000
TECK	0	274	823	2,469	7,407	22,222	66,667	200,000
TGFβ1	0	137	412	1,235	3,704	11,111	33,333	100,000
TRANCE	0	55	165	494	1,481	4,444	13,333	40,000
TremL1	0	55	165	494	1,481	4,444	13,333	40,000
TWEAK	0	27	82	247	741	2,222	6,667	20,000
VEGF-B	0	14	41	123	370	1,111	3,333	10,000
VEGF-R2	0	14	41	123	370	1,111	3,333	10,000

VII. System Recovery

The antibody pairs used in the kits have been tested to recognize their specific antigen. The spiking recovery rate of the cytokines by the kits in serum and cell culture media can be found in their individual manuals.

VIII. Quantibody® Q-Analyzer

Quantibody Q-Analyzer is an array specific, Excel-based program. However, it is not a simple calculation macro as it contains sophisticated data analysis.

Key features:

- *Simplicity:* Easy to operate and requires no professional training. With a simple copy and paste process, the cytokine concentration is determined.
- *Outlier Marking & Removing:* The software can automatically mark and remove the outlier spots for more accurate data analysis
- *Normalization:* The program allows for intra- and inter-slide normalization for large number of samples.
- *Two Positive Controls*: The program takes the two positive controls in each array for normalization.
- *Two Analytical Algorithms*: Users can choose either linear regression or log-log algorithms to meet their analytical needs.
- *Two Data Outputs*: standard curves and digital concentration.
- *User Intervention:* The program allows for user manual handling of those outliers and other analytical data.
- *Lower and Upper Limits Determination:* The program automatically marks out the values below or above the detection range.
- *Standard Deviation:* The program outputs the standard deviations of the quadruplicate spots for data accuracy.
- *Analytical Tips:* Q-Analyzer analysis tips are included in the program.

IX. Troubleshooting guide

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Ensure sufficient incubation time or change sample incubation to an overnight step
	Too low protein concentration in sample	Dilute starting sample less or concentrate sample
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Handle and pipette solutions more gently; De-gas solutions prior to use
	Arrays are not completely covered by reagent	Prepare more reagent and completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
Poor standard curve	Cross-contamination from neighboring wells	Avoid overflowing wash buffer between wells
	"Comet tail" formation	Air dry the slide for at least 1 hour before usage
	Inadequate standard reconstitution or Improper dilution	Reconstitute the lyophilized standard at room temperature before making serial dilutions. Check pipettes and ensure proper serial dilutions
	Inadequate detection	Increase laser power so the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated
	Use freeze-thawed cytokine standards	Always use a new cytokine standard vial for a new experiment. Discard any leftovers
High background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Minimize dust in work environment before starting experiment
	Slide is allowed to dry out	Take additional precautions to prevent slides from dying out during experiment

X. Select Quantibody Publications

1. Stechova, et al. Influence of Maternal Hyperglycaemia on Cord Blood Mononuclear Cells in Response to Diabetes-associated Autoantigens. *Scandinavian Journal of Immunology*. 2009. 70(2):149-158
2. Willingham, SB et al. NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. *J Immunol.* 2009; 183(3):2008-15
3. El Karim et al. Neuropeptides Regulate Expression of Angiogenic Growth Factors in Human Dental Pulp Fibroblasts. *Journal of Endodontics*, 2009; 35(6): 829-833
4. Souquière S. et al. T-Cell tropism of simian T-cell leukaemia virus type 1 and cytokine profiles in relation to proviral load and immunological changes during chronic infection of naturally infected mandrills (*Mandrillus sphinx*). *J Med Primatol*. 2009; 38(4):279-89
5. Sharma, et al. Induction of multiple pro-inflammatory cytokines by respiratory viruses and reversal by standardized *Echinacea*, a potent antiviral herbal extract. *Antiviral Research*. 2009; 83(2)165-170.
6. Altamirano-Dimas, et al. *Echinacea* and anti-inflammatory cytokine responses: Results of a gene and protein array analysis. *Pharmacuetical Biology*. 2009; 47(6): 500-508.
7. Cheung, et al. Cordysinocan, a polysaccharide isolated from cultured *Cordyceps*, activates immune responses in cultured T-lymphocytes and macrophages: Signaling cascade and induction of cytokines. *Journal of Ethonopharmacology*. 2009; 124(1): 61-68.
8. Du, et al. P2-380: Identification and characterization of human autoantibodies that may be used for the treatment of prion diseases. *Alzheimer's and Dementia*. 2009; 4(4): T484-T484.
9. Van Rossum et al. Granulocytosis and thrombocytosis in renal cell carcinoma: a pro-inflammatory cytokine response originating in the tumour. *Neth J Med*. 2009; 67(5):191-4.
10. Zhai, et al. Coordinated Changes in mRNA Turnover, Translation, and RNA Processing Bodies in Bronchial Epithelial Cells following Inflammatory Stimulation. *Molecular and Cellular Biology*. 2008; 28(24): 7414-7426.
11. Gao, et al. A Chinese herbal decoction, Danggui Buxue Tang, activates extracellular signal-regulated kinase in cultured T-lymphocytes. *FEBS Letters*, 2007; 581(26): 5087-5093. (This reference validates mulitplex ELISA results for several analytes with standard ELISA test results).
12. Piganelli, et al: Autoreactive T-cell responses: new technology in pursuit of an old nemesis. (Editorial Review) *Pediatric Diabetes* 2007: 8: 249–251

XI. Experiment Record Form

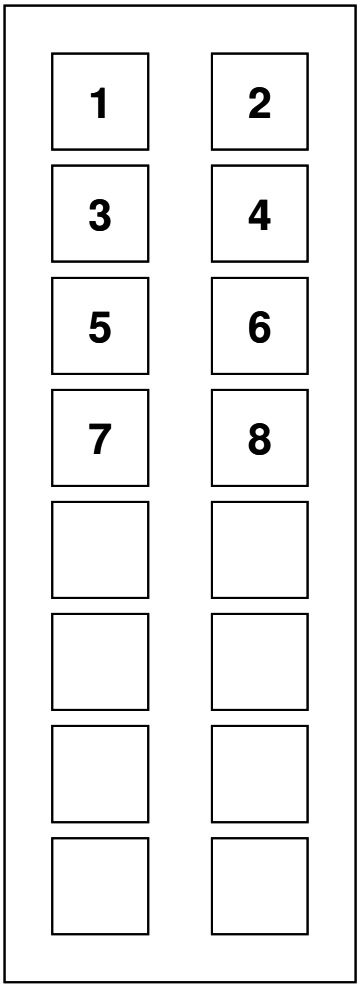
Date: _____

File Name: _____

Laser Power: _____

PMT: _____

Well No.	Sample Name	Dilution factor
1	CNTRL	
2	Std7	
3	Std6	
4	Std5	
5	Std4	
6	Std3	
7	Std2	
8	Std1	
9		
10		
11		
12		
13		
14		
15		
16		



Note:

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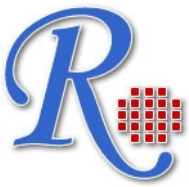

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